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EXPERIMENTS IN VACCINATION AGAINST ANTHRAX.[1]

By ADOLPH EICHHORN, *Chief of the Pathological Division.*

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PREVALENCE OF ANTHRAX AND METHODS OF CONTROL.

Anthrax is a disease that is widely spread throughout the world, and in the United States it is being recognized as one of the most destructive scourges of live stock. In certain sections it is more prevalent than in others, particularly in the Southern States, and since no determined effort has been made toward its suppression it appears to be on the increase, its presence now being recorded in localities where it has never before been recognized.

As the spores of the causative agent of anthrax retain their virulence and remain lodged in the soil in an active state for many years in the infected localities, it is very difficult to prevent the spread of the infection, and the eradication of the disease is thereby rendered a most serious problem.

Various factors have to be considered in the prophylactic control of anthrax, such as the prevention of the continued impregnation of

[1] The author was ably assisted in the technical part of this work by Dr. Raymond A. Kelser, assistant in pathology and bacteriology, to whom credit is due for the painstaking and careful execution of the details of the experiments.

NOTE.—This bulletin is of interest to stock raisers and veterinarians generally.

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the soil with the virus by the proper disposition of the carcasses of animals that have died of the disease, the destruction of the virus contained in the soil by its proper drainage and cultivation, and the prevention of outbreaks through the immunization of the susceptible animals.

In order to attain the greatest success in the control and eradication of the disease, it would appear that the best results can be accomplished only through proper attention to all of the above factors. The execution of these measures would require the earnest cooperation of the stock owners, but even then, on account of the peculiar geographical conditions of certain parts of the country, the drainage and cultivation of the land would not always be feasible, and our efforts must therefore be directed principally toward the sanitary measures and protective vaccination. The enforcement of proper sanitary police regulations in connection with the control of anthrax would no doubt effect a material reduction of the disease, but unfortunately it is rather a difficult task to obtain the cooperation of the interested parties.

The proper disposition of the infective material, particularly the carcasses, should be considered of the utmost importance, since such material constitutes the greatest source of danger toward the spreading of the disease. Drainage from the soil polluted by infected carcasses may carry the infection to distant points and deposit the spores over large areas hitherto uninfected. Buzzards and other birds (Dalrymple), dogs, and even flies may also carry the infection from such sources into uninfected localities. Therefore, in an effort to control the disease, an educational propaganda must be carried out and stringent compulsory measures adopted for the proper disposition of the infective material from premises where the disease appears among the stock.

PROTECTIVE VACCINATION.

A material reduction and a checking of the disease may be successfully accomplished by periodical vaccination of all stock in infected localities. This method, even if practiced alone, would have splendid results in minimizing the losses from the disease in anthrax localities. However, such vaccination must be carried out regularly and irrespective of whether the disease has already appeared on the premises.

Fortunately we have at our command various methods of vaccination which have proved highly efficient in the production of immunity from anthrax. As a matter of fact, this was one of the first infectious diseases in which protective vaccination was successfully demonstrated, and we are indebted to Pasteur for devising the pro-

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cedure of the vaccination for this purpose. Pasteur proved that anthrax bacilli when cultivated at a temperature of from 42° to 43° C. will gradually lose their virulence, and also that when removed from such an attenuating temperature and cultivated under normal incubation temperature they will not change their pathogenicity. Thus cultures attenuated for 24 days will be pathogenic for mice but not for guinea pigs and rabbits, whereas if attenuated for only 12 days at the higher temperature they will be virulent for mice and guinea pigs but not for large rabbits. The attenuated cultures will retain their reduced virulence under ordinary conditions, and only in very exceptional instances has any increase of virulence been observed. This characteristic of the anthrax bacillus led Pasteur to employ the attenuated forms of the anthrax cultures for vaccination purposes. Accordingly he prepared a weakened vaccine from cultures which had been attenuated for 24 days (*premier vaccin*), and for a second injection cultures which had been attenuated for 12 days (*deuxième vaccin*). In the epoch-making demonstration at Pouilly le Fort, before a commission appointed by the French Government, he successfully demonstrated its effectiveness on sheep and cattle. In this instance the vaccinated animals withstood the injection of virulent anthrax bacilli, whereas the controls died. Since that time vaccination against anthrax by the Pasteur method has been very extensively employed throughout the world. Many millions of animals have been vaccinated by this method, and the results in general must be considered very favorable.

At the same time it must be acknowledged that in vaccination by the Pasteur method it is essential to have a potent vaccine and one which is properly tested for its pathogenicity. There are disadvantages in this method of vaccination and these must be given due consideration. The unstable keeping quality of the Pasteur vaccine is a very important factor to be considered. Experience in this line has proved that Pasteur vaccine may deteriorate within a very short time after its preparation, and this has also been demonstrated during the work of the Bureau of Animal Industry in the control of the manufacture of biological products, when periodical tests were undertaken with those of various manufacturers. In repeated instances a vaccine proved inert within three months of its preparation. At other times it remained potent for a period of a year. This no doubt is due to the method of preserving and handling the product. When exposed to light and warm temperature it deteriorates very rapdily, and when it is considered that the products of manufacturers may be stored under unfavorable conditions in branch houses and on the shelves in rural drug stores the loss of potency can be readily explained. For this reason it seems wise to reduce the time limit for

the use of Pasteur anthrax vaccine to three months from the date of its preparation.

The injection of an inert product into animals would impart to the stock owners and veterinarians who employ it a false sense of security and would bring this method of vaccination into disrepute. At times no doubt great losses have resulted from the application of inert vaccines.

Other disadvantages of the Pasteur method which must be considered are, first, that it requires two handlings of the animals before immunity is established; second, that the losses from vaccinations are not insignificant; third, that its standardization is not carried out very accurately; and, fourth, that its administration in herds where the disease has already made its appearance is liable to induce the disease, through the reduction of the resistance of the animal during the process of vaccination, and for this last reason it is best adapted for use only with herds in which the disease has not yet appeared.

These deficiencies of the method have been recognized by many investigators, who have endeavored to devise other methods of vaccination, and particular attention has been directed toward the preparation of a spore vaccine, because of its superior keeping qualities. In Russia at the present time the method of Zenkowsky, and in Hungary a spore vaccine prepared by Detre, are being successfully employed; although, aside from their keeping qualities, these products have all the other disadvantages of the Pasteur method. Successful vaccination by spore vaccines was also demonstrated by Nitta, in Japan, and by others. Other means of vaccination with attenuated living cultures, aggressions, dead bacteria, etc., were tried, but proved of no advantage.

Sclavo, Sobernheim, and others have established that injections of increasing amounts of virulent cultures into immune animals produced a serum which has great protective value against anthrax. Such protective serum may be produced in the various susceptible animals.

PRODUCTION OF SERUM.

The animals which are selected for the preparation of serum are subjected to a preliminary treatment either by sero-vaccination or by Pasteur's method, then at certain regular intervals they are infected with increasing doses of virulent anthrax cultures. For this purpose they receive in about 10 to 14 days following the preliminary treatment an injection of from 0.005 to 0.001 of a loopful of virulent culture. In sheep it is advisable to exercise greater care, especially at

the first injection of virulent material, when a very small quantity of culture should be employed, whereas in cattle and horses it is not necessary to employ less than 0.005 of a loopful. The first injection of virulent culture is usually followed by a considerable reaction, inasmuch as the animals usually develop a febrile condition which persists for several days. The subsequent inoculations are then carried out at intervals of from 2 to 3 weeks in such a way that the dose is soon increased to a loopful, then to several loopfuls, and gradually to several agar cultures, and, finally, to an injection consisting of several large mass cultures. This is quite easily accomplished in cattle and horses, and in 3 to 4 months the animals may become so tolerant to this injection that they will withstand the subcutaneous inoculations of two to three mass cultures without noteworthy reaction. At times considerable extensive local infiltration may follow the injection, which, however, retrogresses within a short time and the general condition of the animals is only slightly influenced. In sheep the immunization causes greater difficulties on account of a greater susceptibility of these animals, and it is difficult to prevent a very small percentage of the animals which are being used for serum production from dying in the course of the hyperimmunization. Nevertheless it is possible, even in sheep, to produce such an immunity that they will withstand the injection of several mass cultures without reacting.[1]

The more virulent the strain of the anthrax culture which has been used for the treatment of the animals the more care must be exercised in the course of the hyperimmunization, but in that case the anthrax serum would also be more potent. Therefore, it is advisable to use anthrax strains which have been recently obtained from fatal infections. It is also advisable to use strains of different origin for the immunization. It is immaterial whether bouillon cultures are used or suspensions from agar cultures, but it is more practical to use the latter method for the inoculating material, since in this instance the quantity of fluid to be injected may be limited to a relatively small amount. Quantities of 500 to 1,000 c. c. of the bouillon cultures cause, as can be readily seen, considerable technical difficulty for injection, whereas the suspensions from four or five mass cultures may be readily distributed in 50 to 60 c. c. of fluid. Fresh cultures which have been cultivated for about 24 hours at 37° C. are as a rule more suitable for inoculation, whereas older cultures with pronounced spore formations possess no advantages over the young cultures.

[1] Bureau of Animal Industry Bulletin 137, "Anthrax, with special reference to the production of immunity," by Charles F. Dawson. 1911. See p. 43.

The inoculations should be made subcutaneously. Intravenous injections as first employed by Sclavo are less effective. The potency of the anthrax serum is in no way increased by this method of immunization. Besides there exists the danger of emboli when in the later stages of the immunization process larger amounts of culture material have to be administered. Animals which have been treated with subcutaneous injections will produce finally an anthrax serum of remarkably high potency.

As a rule the animals which have received one to two agar cultures show a specific protective action of their serum, but for practical purposes it is not advisable to use such a serum. Generally only when the animals stand one-half to one mass culture is the potency of the serum sufficiently strong. A similar condition is manifested in animals used for the production of immune serums for other diseases, the individuals showing a varying response to the injection for the production of immune bodies, i. e., an animal will at times produce a potent serum relatively early, whereas another with the same method of treatment will develop a serum of the same potency only after a considerably longer preparatory treatment. Accordingly, from observation it has been noted that sheep produce the most potent serum, and in this species of animals the individual differences are of almost no consequence, so that almost every animal produces a good anthrax serum. Horses also produce a potent serum, although single individuals may show great variations. The anthrax serum from cattle is quite potent, but in its protective value it does not equal horse and sheep serum.

It is best to draw the blood 14 to 16 days after the last injection; an earlier bleeding should be avoided. Not infrequently it occurs that animals after an apparent recovery following the inoculation reaction and after a period in which they are free of fever on the eighth or ninth day suddenly develop a rise in temperature. This has been established by Sclavo and Burow. Then, again, repeated regular blood examinations showed that at this time and even later, up to the tenth and eleventh days following inoculation, occasional anthrax bacilli may appear in the blood of the animals in greater numbers.

The bleeding is carried out in the ordinary way, and the blood is collected in large sterilized glass cylinders or similar receptacles of about 2 or 3 liters capacity. Seven or eight liters of blood may be drawn from cattle, about the same quantity from horses, and about 1 to $1\frac{1}{2}$ liters from sheep. After 2 or 3 days another bleeding is made. In this instance, however, only a small quantity of blood should be drawn. The animals resist these operations very readily, and after a lapse of 14 days they are ready for another injection,

which is then followed in from 14 to 16 days by repeated bleedings. Thus, in the period of a year, the same animals may be bled 10 to 11 times, and such animals can be used in this way for several years, alternating the injections with the bleedings, provided they are kept in a well-nourished and healthy condition.

In order to obtain the largest possible yield of serum from the blood drawn into the glass cylinders a weight is attached to the same and released onto the clotted blood in about 12 hours after being drawn. The diameter of the weight is about half an inch less than the cylinder and its weight is about 2 pounds. In about 24 hours the clear serum is then siphoned into sterile bottles and preserved with 0.5 per cent of carbolic acid. If proper precautions have been practised, it is not necessary to pass the serum through Berkefeld filters; however, if there is the slightest doubt as to its sterility, it is desirable to filter the serum before bottling. It is advisable to distribute the serum in various-sized brown bottles, which should be securely corked and paraffined.

STANDARDIZATION OF THE SERUM.

The testing of the serum must be carried out primarily to determine its potency. It is to be regretted that for this purpose there are no accurate or definite methods known, and it is almost impossible to establish the absolute protective value of the serum, because the animals on which it is being tested are so very highly susceptible to the disease. Nevertheless, it is possible to establish a relative value for all practical purposes through laboratory experiments, and some investigators believe that rabbits are best adapted for the purpose. The standardization test as recommended by Sobernheim is still employed by various investigators. This test is carried out as follows:

Potency test for anthrax serum (Sobernheim).

Rabbit.	First injection.	Second injection.
A	2 c. c. of immune serum (intravenous)	Follow immediately by a subcutaneous injection of 0.001 loopful of a suspension of virulent anthrax bacilli in 1 c. c. of 0.7 per cent sodium-chlorid solution.
B	3 c. c. of immune serum (intravenous)	
C	4 c. c. of immune serum (intravenous)	
D	5 c. c. of immune serum (intravenous)	
E	6 c. c. of immune serum (intravenous)	
F (control)	0.001 loopful of a suspension of virulent anthrax bacilli in 1 c. c. of 0.7 per cent sodium-chlorid solution.	
G (control)	do	

According to extensive experience, a serum is considered potent and satisfactory for immunization purposes when at least two of the five rabbits given the serum remain alive and the others die later than the control animals. Should more than the two animals remain alive, while the control animals die in about 48 hours, the

serum has an extraordinary potency. It should be noted that it does not follow that those rabbits which receive the smallest serum doses should die, since not infrequently they may remain alive when the rabbits receiving larger doses succumb.

This method of standardization has not been proved as accurate and reliable as the test recommended by Ascoli, and which has been employed in the experimental work with serum prepared in connection with our experiments. In this test a 24-hour-old attenuated bouillon culture is used, which is of such virulence that when introduced subcutaneously in a 0.25 c. c. dose into 350-gram guinea pigs it will kill them in from two to three days. These test cultures must be previously standardized in such a way that they will kill guinea pigs which 24 hours previously have been injected intraperitoneally with 2 c. c. of normal serum. Guinea pigs treated in the same manner and with the same dose of titrated standardized immune blood serum must remain alive.

The testing of the serum is carried out on six guinea pigs, each receiving intraperitoneally 2 c. c. of the serum to be tested, and 24 hours later the established dose of the test culture is injected subcutaneously in the axillary region. The serum is considered satisfactory for immunization purposes if at least four of the guinea pigs remain alive over six days while the control animals die within three or four days. For protective and curative purposes in man, only such serum should be selected which, by carrying out the same conditions of the test, protect the guinea pig in 0.5 to 1 c. c. doses.

EXPERIMENTAL DATA.

HYPERIMMUNIZATION OF HORSES.

On September 8, 1914, two horses, Nos. 48 and 96, were vaccinated against anthrax according to Pasteur's method. On September 29 these two horses were given approximately 0.01 of a loopful of virulent anthrax bacilli subcutaneously. Horse No. 48 showed no apparent reaction following the injection. Horse No. 96, however, developed local anthrax at the point of inoculation. The swelling became enlarged and there was a considerable area of edema below the same. This condition persisted for approximately a week, and finally disappeared. The animal, however, showed no appreciable rise in temperature during this period.

The following table gives in detail the process of hyperimmunization:

Hyperimmunization of horses Nos. 48 and 96.

Date.	Amount of virus given each horse.	Result.
1914.		
Sept. 29	0.01 loopful	No apparent reaction in horse 48. Horse 96 developed anthrax at point of inoculation; large swelling; edema of neighboring tissue. Persisted about one week.
Oct. 24	1 loopful	No noticeable reaction in either animal.
Nov. 15	10 loopfuls	Do.
Dec. 9	5 c. c. of an emulsion, representing one-half growth of agar culture.	Horse 48 showed a temperature of 102.2° the following day; horse 96, 101°. Both animals developed a small, hard nodule at point of inoculation.
Dec. 29	20 c. c. of emulsion, representing washing of growth from 2 agar cultures.	Both animals developed small abscess at point of inoculation.
Jan. 19	30 c. c. of emulsion, growth from 8 agar cultures.	No reaction.
Feb. 6	40 c. c. of emulsion, growth from 2 mass cultures from flasks, surface area 6 by 2½ inches.	Slight reaction in horse 96. Horse 48 showed quite an intensive reaction, developing a large swelling at point of inoculation; persisted several days.
Mar. 5	50 c. c. of emulsion, growth from 4 mass cultures from flasks, surface area 6 by 2½ inches.	No apparent reaction.
Mar. 31	50 c. c. of emulsion, growth from 8 mass cultures from flasks, surface area 6 by 2½ inches.	Slight local reaction in each case.
Apr. 19	do	Do.
Apr. 28	do	Slight rise in temperature in both cases.
May 11	do	Slight temperature and local reaction.
May 24	do	Do.
June 12	do	Slight local reaction.

In the above work four strains of anthrax bacilli were used, known to us as "Davis," "6071," "Burt," and "Boener"—the first two strains being highly virulent types and the latter two very much weaker. In all cases where the larger amounts of the virus were given the injections were made at 4 to 6 different points in order to minimize abscess formation.

It might be well also to state here that the irregularity in the time between injections was due to the fact that this work was interfered with by the outbreak of foot-and-mouth disease in this country, and for this reason it was also impossible to subject the blood to periodical tests to ascertain its immunizing value at the different intervals between injections. Experience proved that horses may produce highly potent serum following the injection of the first or second mass cultures. It is therefore advisable to subject the blood of the animals to periodical tests for potency throughout the course of immunization.

On June 25, 1915, 6 liters of blood were drawn from each horse into the glass bleeding cylinders previously described. Since this date these animals have been bled regularly, 6 liters being taken from each horse, and an injection of virus made in the intervals between bleedings.

SERUM TESTS.

In standardizing our serum, that taken from each horse was tested separately. The following procedure was carried out: Three series

of guinea pigs were inoculated intraperitoneally with varying amounts of serum, and 48 hours later were injected with 0.25 c. c. of a 24-hour bouillon subculture of an attenuated strain known as "Davis D." This culture had been attenuated by growing it at a temperature of 42°–43° C. for a period of 20 days. Previous tests of this culture showed that it was uniformly pathogenic for guinea pigs, killing them in two to three days, but it failed to kill rabbits. The results of this test are contained in the following table:

Standardization tests of anthrax serum (serum injected intraperitoneally; virus 24 hours later subcutaneously).

SERUM 48.

Guinea pig No.	Amount of serum.	Amount of virus.	Result.
1	1.0 c. c.	0.25 c. c.	Remained alive.
2	1.5 c. c.	0.25 c. c.	Died on third day.
3	2.0 c. c.	0.25 c. c.	Remained alive.
4	2.5 c. c.	0.25 c. c.	Do.
5	3.0 c. c.	0.25 c. c.	Do.
6	3.5 c. c.	0.25 c. c.	Do.

SERUM 96.

1	1.0 c. c.	0.25 c. c.	Remained alive.
2	1.5 c. c.	0.25 c. c.	Do.
3	2.0 c. c.	0.25 c. c.	Died on third day.
4	2.5 c. c.	0.25 c. c.	Remained alive.
5	3.0 c. c.	0.25 c. c.	Do.
6	3.5 c. c.	0.25 c. c.	Do.

NORMAL HORSE SERUM.

1	1.0 c. c.	0.25 c. c.	Died on fourth day.
2	1.5 c. c.	0.25 c. c.	Died on third day.
3	2.0 c. c.	0.25 c. c.	Died on fourth day.
4	2.5 c. c.	0.25 c. c.	Remained alive.
5	3.0 c. c.	0.25 c. c.	Died on fourth day.
6	3.5 c. c.	0.25 c. c.	Died on third day.

In view of these results it was decided to use the "Davis D" culture in the preparation of our spore vaccine, to be used simultaneously with the serum.

Extensive tests to determine whether or not the immune serums possessed a bactericidal property proved negative.

PREPARATION OF SPORE VACCINE.

The four cultures used for the hyperimmunization of the horses were attenuated at a temperature of 42.5° C. for varying periods. From time to time they were tested for their pathogenicity by inoculation into mice, guinea pigs, and rabbits. The cultures, which were removed from the incubator after 20 days of attenuation, proved satisfactory for the purpose, inasmuch as the test inoculation demon-

strated their virulence for the mice and guinea pigs, but not for rabbits.

For the purpose of producing a spore vaccine it is desirable to use a peptone-free agar media and after inoculation with the attenuated culture to grow the organism at a temperature of 37.5° C. for 4 to 7 days, by which time an abundance of spores will have formed. The growth is then washed from the slants and collected in a sterile flask and heated at a temperature of 60° C. for one-half hour, to destroy the vegetative forms of the organism. A measured quantity of this suspension can then be plated out in the usual manner and the spore content of 1 c. c. of the suspension established. A dilution can then be made to the desired amount for inoculation purposes. Thus, if it is desired to use for vaccination 1,000,000 spores, it is best to dilute the vaccine to a quantity of which 1 c. c. would contain this number. Of such vaccine 1 c. c. would constitute the dose for cattle and horses, with correspondingly smaller doses for calves and sheep.

In all forms of vaccination against anthrax in sheep the greatest care must be exercised, since these animals are very susceptible to the disease, and at times vaccines which have no ill effects on cattle will prove fatal to sheep; therefore the dose of the spore vaccine for sheep should not be more than one-fourth the amount given cattle.

In the preparation of spore vaccines it is essential to submit every lot to a test for pathogenicity by inoculating approximately 250,000 spores—that is, 0.25 c. c. of the standard suspension—into guinea pigs and rabbits before employing the same for vaccination purposes. The guinea pigs should die in from 2 to 5 days, whereas the rabbits should remain alive.

In consideration of the keeping qualities of the spore vaccine, large lots can be prepared without fear of deterioration. In the bottling and storing of the same, however, proper care should be taken to prevent contamination.

TECHNIC OF ADMINISTRATION.

For immunization purposes by the simultaneous method the serum should be injected first. It is desirable to divide the herd into groups of 10 or 12 and inject first each animal of the group with the serum, following this with the injection of the spore vaccine. The serum should be injected on one side, either on the neck or back of the shoulder, and the spore vaccine on the other side, the injections being made subcutaneously.

In herds where the disease has already made its appearance it is necessary to take the temperatures of all the animals and to subject to the simultaneous vaccination only those that show no rise in tem-

perature. All others should be given the serum-alone treatment in doses varying in accordance with the severity of the symptoms manifested by the individual animals. If the examination reveals a considerable number of infections, it is advisable to use the serum alone for all the animals, and in 3 or 4 weeks to revaccinate by the simultaneous method.

The dosage should depend on the potency of the serum, serum of a high potency naturally being most desirable; thus, in some instances serum in 5 c. c. doses for large animals and 3 c. c. for smaller ones was found to be effective for immunization purposes. Unfortunately all hyperimmune animals do not yield serum of such high potency, and for this reason it is obvious that accurate potency tests should be carried out by the producer of the serum.

In the treatment of anthrax, serum should be administered in large doses. An animal showing only a high temperature, with no other manifestations of the disease, should be given from 30 to 50 c. c., but if the gravity of the disease is pronounced, 100 c. c. should be administered. In almost every instance a drop in temperature may be observed and a diminishing of the severity of the symptoms. At times, however, a relapse occurs about the second or third day following the serum injection, when it becomes necessary to administer another dose of serum. It has been proved that animals affected with anthrax, even after the bacilli are found in the blood circulation, may recover after an injection of potent serum.

The simultaneous treatment, as in the Pasteur treatment, may at times result in a temperature and systemic reaction in the animals. These manifestations are indicated by an elevation of temperature and sometimes by a swelling at the point of inoculation of the spore vaccine. These symptoms, however, are usually of short duration, and only in very exceptional cases will they result in the loss of the animal. However, if the reaction following the injection of the spore vaccine threatens the life of the animal, a second injection of serum should be administered.

The anthrax serum injected simultaneously with the vaccine has a counteracting effect upon the reaction which may follow the injection of the spore vaccine during the process of immunization.

At times anaphylactic reactions are observed as a result of the serum injected, especially in cases where the serum is foreign to the animals treated. These manifestations appear as a rule within one-half hour after injection, in the form of urticarialike eruptions, swelling of the head, slight chills, and rise in temperature. More severe symptoms have also been noted to follow such injections, but they almost invariably subside within a few hours.

TEST OF THE SIMULTANEOUS METHOD ON CATTLE AND SHEEP.

A series of experiments was conducted at the experiment station of the Bureau of Animal Industry at Bethesda, Md., to establish the efficiency of the simultaneous method of anthrax immunization on cattle and sheep.

For this purpose 6 head of cattle and 5 sheep were given the simultaneous injection of anthrax serum and spore vaccine. Three weeks subsequent to immunization they were subjected to infection tests which consisted of a subcutaneous administration of 0.25 c. c. for the cattle and 0.125 c. c. for the sheep of blood from a guinea pig which had died from an artificial infection with our most virulent strain of anthrax.

The microscopic examination of the blood of the guinea pig showed it to be heavily charged with anthrax bacilli, but in order to make the test as severe as possible it was deemed advisable to use such excessive amounts. Three additional cattle and two sheep were used as checks, receiving only the virulent blood. As a result of this infection all animals manifested an elevation of temperature ranging from 103° to 107° F. The control animals especially were markedly affected with typical manifestations of anthrax and all succumbed within two to eight days following infection. All but one of the vaccinated sheep succumbed to anthrax, but at a later date than the check animals. Of the immunized cattle a marked temperature reaction was noted, but all of these animals recovered with the exception of a small, undersized, weak calf, which died in six days following infection.

While in the above test the sheep succumbed and one of the small calves died of anthrax, nevertheless the potency of the serum was demonstrated. The excessive virulent blood used for the infection was extraordinary and could not be compared with the amount of virus taken by a susceptible animal in cases of natural infection.

FIELD TESTS.

On June 21, 1915, Dr. R. R. Ashworth, a dairy inspector for the District of Columbia, notified our office that a number of deaths among hogs were occurring on a farm in Maryland, just outside of the District. The symptoms described by Dr. Ashworth pointed suspiciously to anthrax. A visit was made to the farm the same morning, and after an autopsy on several animals, followed by a bacteriological examination, a definite diagnosis of anthrax was established. This was later conclusively verified by animal inoculation tests.

At that time 7 shoats and 4 sows had died of the disease and 3 shoats, 4 sows, and 1 boar were showing symptoms of anthrax, several of the

sick animals manifesting the characteristic edema of the throat region. It is desired to make particular mention of the boar, a fine pure-bred animal, which was in an almost comatose condition, showing a profuse bloody diarrhea, and a temperature of 106° F. One of the sows was also in a very critical condition.

On the afternoon of June 21 the affected animals were given injections of the immune serum, the boar receiving 100 c. c., the sows 50 c. c., and the shoats 30 c. c. On the following day a visit was made to the farm to immunize the remaining hogs, which as yet had shown no symptoms of the disease. A total of 138 were given protective doses of the serum, the larger hogs weighing 75 pounds or over receiving 10 c. c. and the smaller animals 5 c. c. Marked improvement was noted in the sick animals that had been treated the day before.

On June 23 another visit was made to the farm. All of the sick animals showed still further improvement. The boar was given 60 c. c. more of immune serum and the sow that had been the most sick was given an additional 30 c. c.

The result of this work was that every affected animal recovered, and up to the present time not a single death from anthrax has been reported in those animals that received portective doses of the serum.

In the early part of July an outbreak of anthrax was reported from Queen Anne County, Md. On July 13 two inspectors from the bureau were detailed to make an investigation, with a view to using our immune serum and spore vaccine in an effort to control the outbreak. The disease had made its first appearance about a month previous to this time, when a farmer lost a cow from anthrax. A few days later a neighbor on an adjoining farm lost a hog from the disease. Following this, the disease made its appearance on five other farms in the immediate vicinity, the greater percentage of animals stricken dying of the apoplectic form of the malady. Animals on some of the farms had been treated with single injections of a commercial vaccine before the arrival of our inspectors. Immunization tests were at once started with the bureau serum and spore vaccine, with the following results:

The animals on six farms where losses had occurred from anthrax were vaccinated, the cattle, horses, and mules receiving 10 c. c. each of serum and 1 c. c. of spore vaccine, except, however, in cases where there was reason to believe an animal might be in the incubative stage of the disease, when the vaccine was omitted and the dose of serum increased. Sheep and hogs on the infected farms were given the serum-alone treatment, receiving from 5 to 10 c. c. each.

On the day subsequent to vaccination a mule on one of the farms showed symptoms of anthrax, there being an elevation of temperature and a characteristic swelling on one side of the neck, the side

opposite to where the vaccine had been injected. This animal was given an injection of 60 c. c. of serum and made a speedy recovery.

In all, 399 animals, including horses, mules, cattle, sheep, and hogs on farms where the disease had broken out, were treated with the bureau serum and vaccine. Previous to this an aggregate of 10 cattle, 3 mules, and 13 hogs had died of anthrax on these farms. On the morning of the day following vaccination a cow on one of the farms died of anthrax. Exclusive of the above, no losses from anthrax have occurred on any of these farms.

Approximately 140 animals on several other infected farms were vaccinated with a commercial vaccine by a representative of the State live stock sanitary board. Within a day or two following this vaccination it was reported 3 cows and 1 mule died of anthrax, and since then 2 more cows have died of the disease.

Another opportunity was afforded us to test the serum and vaccine in an outbreak of anthrax in Noxubee County, Miss., where a number of farms were reported to be infected with the disease. A quantity of serum and spore vaccine was furnished, and an inspector detailed from the bureau station at Birmingham, Ala., to conduct the work. On various farms where the disease had made its appearance a total of 125 cattle were given the simultaneous treatment. In addition 3 animals which showed symptoms of the disease were given 30 c. c. of serum alone. No deaths from anthrax occurred immediately following or since the vaccination, the affected animals having all recovered from the disease.

USE OF SERUM IN TREATMENT OF ANTHRAX IN MAN.

Extensive data are available on the effectiveness of anthrax serum for the treatment of the disease in man. It is recommended that from 30 to 40 c. c. of serum be injected in three or four different places. Should no improvement follow in 24 hours an additional injection of 20 to 30 c. c. of serum should be administered.

In most instances the results are very favorable, and this treatment is acknowledged to be superior to any other mode of treatment known for this disease.

CONCENTRATION OF SERUM.

Experiments are now being conducted in drying immune serum with a view to preparing the same in pellet form. For this purpose the serum has been dried in shallow pans in a serum-drying apparatus. After thorough drying it is scraped from the pans, milled into a fine powder, and prepared in a pellet machine into proper-sized pellets. The spore vaccine is also being prepared in a similar

manner. This procedure would greatly simplify the administration of the serum and vaccine and, besides, the products would be in a form least likely to deteriorate or become contaminated.

The proteids containing the protective bodies of the serum have also been successfully precipitated through fractional saturation of the serum with ammonium sulphate, and further work along this line is now being conducted. However, this work and the work on the drying and concentration of the products are still in the experimental stage, and it is our aim to properly work out a method most suitable for immunization of animals in the field.

CONCLUSION.

1. Horses are suitable for the production of highly potent anthrax serum. Serum of such horses should protect large animals in 10 c. c. doses.

2. The use of the serum-alone treatment is indicated in cases where the infection has already occurred in a herd. Since the serum confers only a passive immunity, it is advisable to revaccinate the herd in from three to five weeks by the simultaneous method.

3. The serum possesses great curative value. Depending on the severity of the infection, the curative dose is from 30 to 100 c. c.; the injection to be repeated if necessary.

4. For the simultaneous treatment a spore vaccine, carefully standardized, is preferable to the ordinary Pasteur vaccine.

5. Spore vaccine should be employed also in preference to the Pasteur vaccines for immunization with vaccine alone. This vaccine has a decided advantage over the Pasteur, because of the possibility of more accurate dosing and because of its better keeping qualities.

6. Experiments with concentrated serum and dry spore vaccine are very promising. This method would greatly simplify the vaccination process and also insure the product against subsequent contamination and deterioration.

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